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THE SPERMATOZOA OF THE ONE-PALPED SPIDER *TIDARREN ARGO* (ARANEAE, THERIDIIDAE)

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ABSTRACT. The species of the genus *Tidarren* are known for their one-palped males and outstanding copulatory behavior. In our ultrastructural observations of *T. argo* Knoflach & van Harten 2001, we show that this species possesses highly specific spermatozoa which differ from those found in other spiders: The nucleus of the sperm cell is strongly elongated and characterized by a conspicuous implantation fossa. The basis of the axoneme is located close to the acrosomal complex. The axoneme starts in front of the implantation fossa which extends deeply into the postcentriolar elongation. The implantation fossa is filled with dense staining globules and granules as in other theridiid species. Apart from these peculiarities, in *T. argo* the proximal centriole is located extraordinarily far away from the distal one. The encapsulated cleistospermia are surrounded by a thin secretion sheath. Remarkably, mature spermatozoa are not densely packed, but embedded in a copious secretion.

Keywords: Spider sperm ultrastructure, nucleus, implantation fossa, centriole, secretion

The theridiid spider *Tidarren argo* which was first described by Knoflach & van Harten 2001 from Yemen exhibits several peculiarities in exomorphology and behavior. The males amputate one palp some hours after the penultimate molt. Such self-amputation is known only from *Tidarren* and *Echinotheridion* species, but from no other spider (Knoflach & van Harten 2000, 2001; Knoflach 2002). The reason for palp removal may be to increase locomotor performance as shown for *T. sisyphoides* (Walckenaer 1842) (see Ramos et al. 2004). In *T. argo*, the male dies during copulation and even becomes emasculated by the female. Immediately after insertion the female twists off the single male palp, which then continues with sperm transfer disconnected from the male (for details see Knoflach & van Harten 2001). Based on these outstanding features, the present study focuses on the fine structure of the spermatozoa, which are briefly compared with our own unpublished observations on other theridiid spiders.

Tidarren argo from Yemen, Khamis Bani Sa'd, 15°11′N 43°25′E, were kept alive in Innsbruck, in plastic boxes at room temperature. From this breeding stock, male specimens were dissected and fixed in 3.5% glutaraldehyde in 0.1 M phosphate buffer, followed by postfixation in buffered 2% os-

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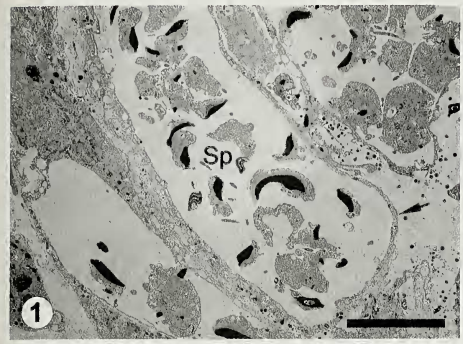
Figures 1–4.—Late-stage spermatids in *Tidarren argo*. 1. Overview of part of the testis. Spermatids grouped together in cysts which are surrounded by extensions of the somatic cells (arrow). Scale bar = 10 μm. 2. Longitudinal section of two spermatids. The ribbon-shaped nucleus coils several times around the axoneme as evident on the right spermatid. Arrow points to nuclear pores. Scale bar = 1 μm. 3. The nucleus is surrounded by a manchette of microtubules. Nuclear canal runs along outer edge of the nucleus. The axoneme possesses a 9 × 2 +3 microtubular pattern. Scale bar = 0.5 μm. 4. Longitudinal section of spermatids. Note the aberrant organization: Axonemal basis (dC) located in front of implantation fossa near acrosomal vacuole; implantation fossa with granular dense material; nucleus strongly elongated, its anterior part triangular. Scale bar = 1 μm. Abbreviations: AV = acrosomal vacuole, Ax = axoneme, dC = distal centriole, Fl = flagellum, IF = implantation fossa, M = mitochondria, Mm = manchette of microtubules, N = nucleus, NC = nuclear canal, Sp = spermatozoa, V = vesicles.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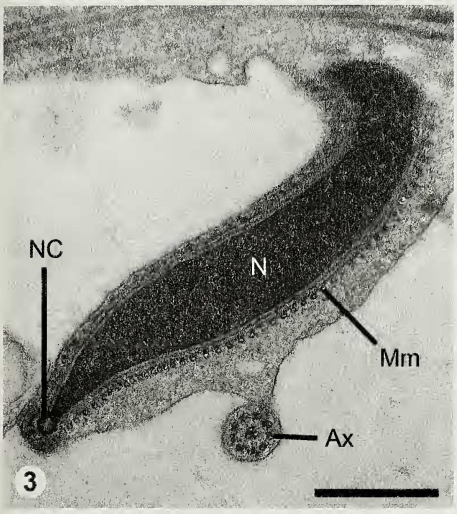
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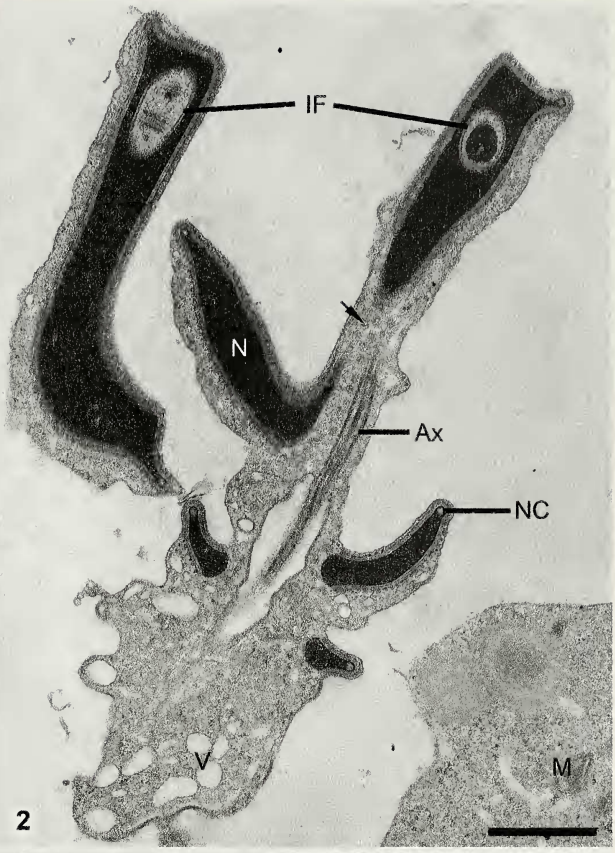
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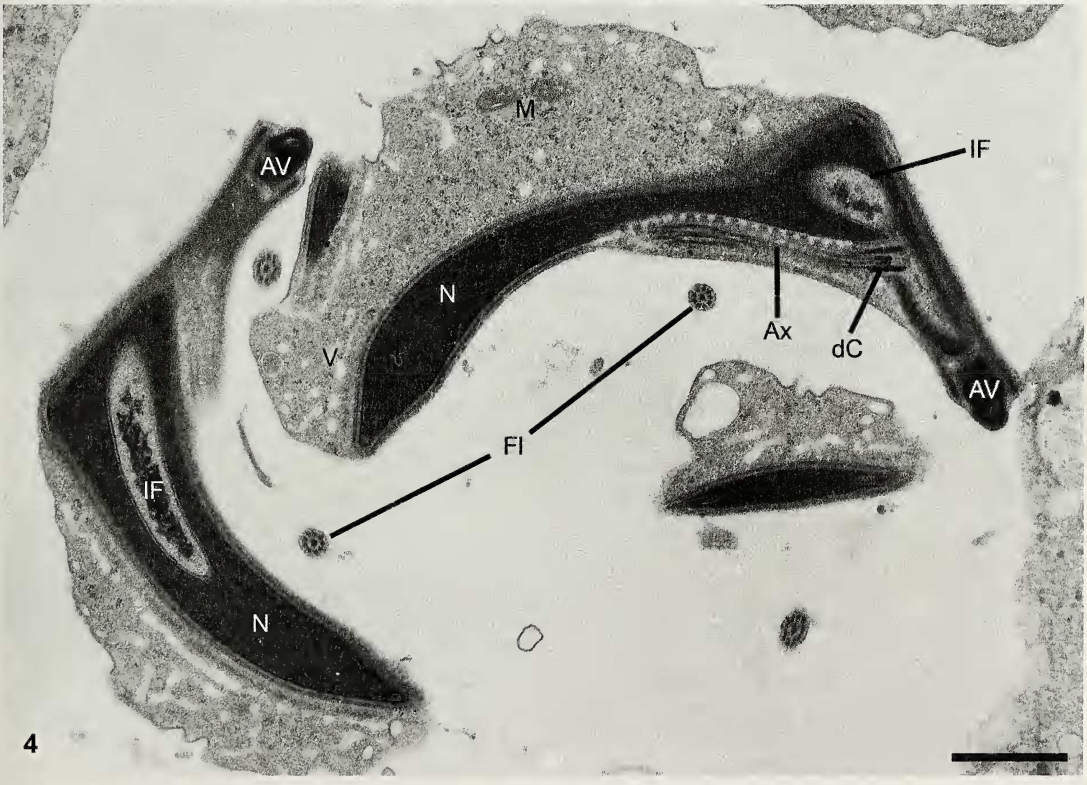
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mium tetroxide. After washing, the specimens were rinsed in graded ethanol solutions (60%, 70%, 80%, 96%, absolute) and embedded in Spurrs resin (Spurr 1969). Ultrathin sections were made with a Leica ultramicrotome and stained with uranyl acetate and lead citrate (Reynolds 1963). Examination was performed with a Zeiss EM 10A electron microscope. For depository of voucher specimens see Knoflach & van Harten (2001).

Differentiation of the spermatozoa takes place in cysts which are surrounded by extensions of the somatic cells. These are located in the periphery of the relatively small testes. The spermatids are loosely distributed within these cysts (Fig. 1). In the following account, the shape of the main cell components of late-stage spermatids will be described, because this stage is most useful for comparative spermatological studies. A reconstruction of a late-stage spermatid is given in Fig. 5.

Nucleus.—In late-stage spermatids the nucleus is the most prominent component. It is strongly elongated and turns several times around the axoneme (Fig. 2). In cross-sections the main part of the nucleus forms a flattened ribbon (Figs. 2, 3). Only the anterior part is more or less lens-shaped, containing the axonemal basis and the implantation fossa (Fig. 4). Until the coiling process, the nucleus is surrounded by a manchette of microtubules (Fig. 3). In longitudinal sections the anterior part of the nucleus forms a triangle with the main part (postcentriolar elongation, see below) (Fig. 4). Along the outer edge of the nucleus runs a nuclear canal, which contains the acrosomal filament in its anterior part (Figs. 2, 3, 9).

Implantation fossa and axoneme.—During spermatogenesis normally an indentation of the nucleus is formed in front of the axonemal basis, the so-called implantation fossa. In *T. argo* the axonemal basis migrates to the anterior part of the nucleus and is finally located close to the acrosomal vacuole (Fig. 4). The implantation fossa extends behind the axonemal basis deeply into the postcentriolar elongation of the nucleus, which in *T. argo* constitutes the main part of the nucleus (Figs. 4, 5). Within the implantation fossa several globules and granules are accumulated which are very dense in mature spermatozoa (Figs. 6, 7). Embedded in this material, the proximal centriole is located extraordinarily far away from the distal one (Figs. 6, 7). A reconstruction of a longitudinal section of the anterior part of a late-stage spermatid is given in Fig. 12. The axoneme possesses the 9 × 2 + 3 pattern of microtubules (Fig. 3).

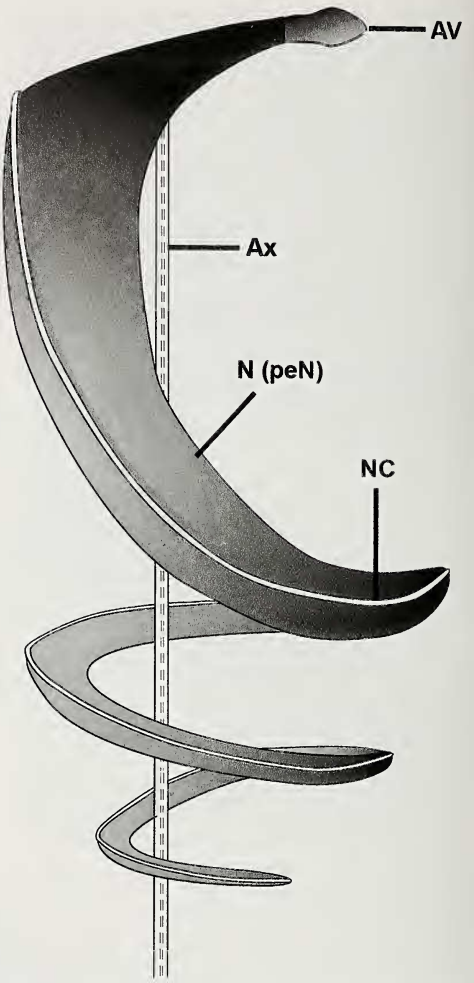
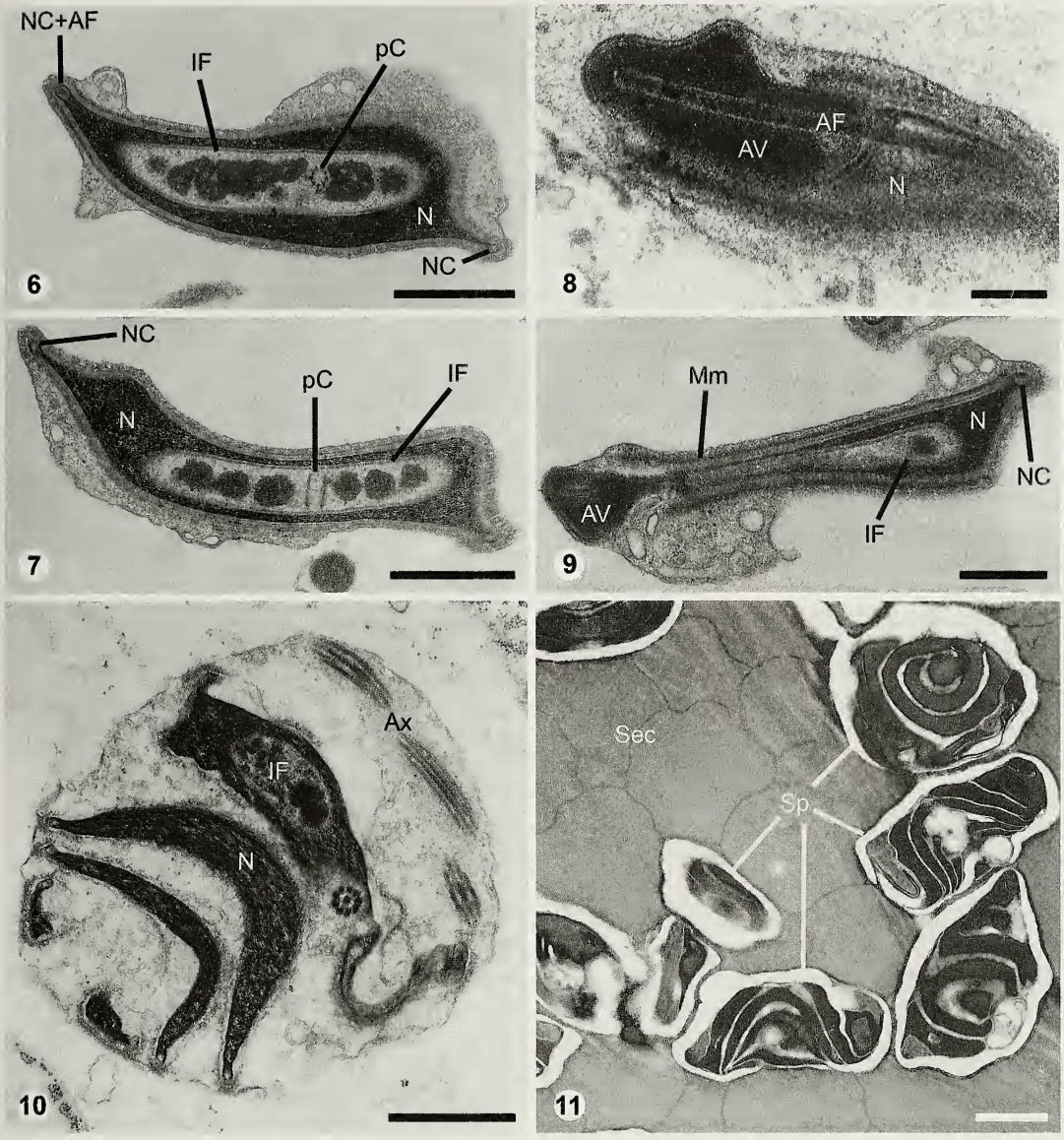


Figure 5.—Schematic reconstruction of a late-stage spermatid of *Tidarren argo* (only main cell components shown). Note the elongated nucleus coiling several times around the axoneme. As a consequence of the extremely positioned axonemal basis close to the acrosomal vacuole the main part of the nucleus (behind the axonemal basis) can be determined as postcentriolar elongation of the nucleus (peN). Abbreviations: AV = acrosomal vacuole, Ax = axoneme, N (peN) = nucleus (postcentriolar elongation of the nucleus), NC = nuclear canal.

Acrosomal complex.—The acrosomal vacuole has an irregular arrowhead-shape (Fig. 8). The basis of the acrosomal vacuole is very



Figures 6–11.—Late-stage spermatids and mature spermatozoa of *Tidarren argo*. 6, 7. Sections of spermatids in the region of the implantation fossa. Note the proximal centriole located deeply within the implantation fossa which is filled with globular and granular dense material. Scale bars = 1 μm. 8. Longitudinal section of the irregularly shaped acrosomal vacuole. Acrosomal filament starts at anterior part of acrosomal vacuole and continues into nuclear canal. Scale bar = 0.25 μm. 9. Front part of spermatid. Note irregular shape of acrosomal vacuole. Manchette of microtubules around nucleus continues to acrosomal vacuole. The acrosomal filament seems very short, because of the empty nuclear canal close to the acrosomal vacuole. Scale bar = 0.5 μm. 10. At the end of spermatogenesis the main cell components (nucleus, axoneme and acrosomal vacuole) coil within the cell. Scale bar = 1 μm. 11. Section of spermophore of palpal organ. Mature spermatozoa possess a thin secretion sheath and are embedded in a dense conspicuous secretion. Scale bar = 1 μm. Abbreviations: AF = acrosomal filament, AV = acrosomal vacuole, Ax = axoneme, pC = proximal centriole, IF = implantation fossa, Mm = manchette of microtubules, N = nucleus, NC = nuclear canal, Sec = secretion, Sp = spermatozoa.

thin. A layer of dense material is located between the acrosomal vacuole and the nucleus (Fig. 9). The manchette of microtubules surrounds more than half of the acrosomal vacuole (Fig. 9). The subacrosomal space is narrow and contains the acrosomal filament which continues into the nuclear canal (Fig. 8, 9). The short acrosomal filament ends near the axonemal basis (Fig. 9).

Additional cell components.—Other cell components, e.g., mitochondria, Golgi apparatus, and vesicles are mainly seen in the cytoplasm of the posterior part of the spermatid. They seem to be absent in mature spermatozoa.

Mature spermatozoa.—At the end of spermatogenesis the spermatids coil. The main cell components (acrosomal vacuole, nucleus and axoneme) are involved in this coiling process within the sperm cell. The nucleus coils up to four times and the axoneme coils at the periphery of the cell (Fig. 10). Finally, in mature spermatozoa, which receive a secretion sheath, cell components are compact and tightly together (Fig. 11). The secretion sheath is rather thin and the mature spermatozoa are embedded in a conspicuous, dense secretion which apparently hinders the fixation process during preparation as seen in Fig. 11. A reconstruction of a section of a mature spermatozoon is given in Fig. 13. Interestingly, the mature spermatozoa are not densely packed in the spermophore of the palpal organ (Fig. 11).

The spermatozoa of *Tidarren argo* possess a highly derivative organization with most aberrant features in comparison to other spider species (e.g., Ōsaki 1969, 1972; Reger 1970; Boissin 1973; Alberti & Weinmann 1985; Alberti et al. 1986; Alberti 1990; Alberti & Coyle 1991; Michalik et al. 2003). Unfortunately, no other investigations dealing with fine structure of theridiid spermatozoa exist to allow an evaluation of our results. Hence, we compare the results mainly with our personal observations on other theridiid spiders (PM pers. obs.). On this evidence it appears that the spermatozoa of *T. argo* must be regarded as highly specialized, both within Theridiidae and within spiders in general. The nucleus of the *T. argo* spermatozoa is strongly elongated and ribbon-shaped over most of its length. As a consequence of the unusual position of the axonemal basis close to the acrosomal vacuole, the main part of the nucleus is represented

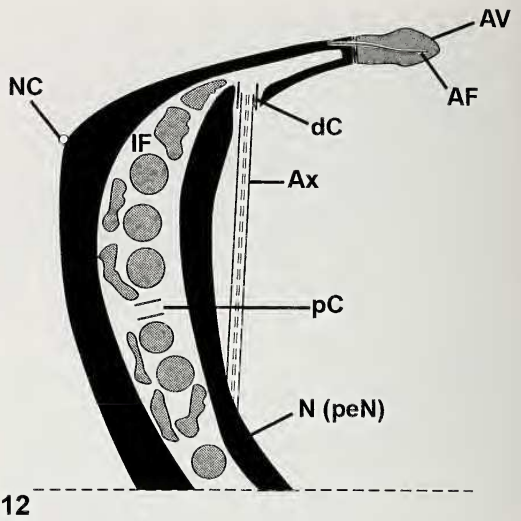


Figure 12.—Schematic reconstruction of a longitudinal section of the front part of a late-stage spermatid in *Tidarren argo*. Note the proximal centriole which is embedded in the globular and granular dense material within the implantation fossa. The acrosomal filament extends only into the most anterior part of the nuclear canal. Abbreviations: AF = acrosomal filament, AV = acrosomal vacuole, Ax = axoneme, IF = implantation fossa, dC = distal centriole, pC = proximal centriole, N (peN) = nucleus (postcentriolar elongation of nucleus), NC = nuclear canal.

by the so-called postcentriolar elongation of the nucleus. In contrast, in our observations on *Argyrodes argyrodes* (Walckenaer 1842), *Crustulina guttata* (Wider 1834), *Nesticodes rufipes* (Lucas 1846), *Steatoda grossa* (C.L. Koch 1838) and *Theridion nigrovariegatum* Simon 1873, the nucleus is completely different, oval in cross section and more compact. The most striking features are the position of the axonemal basis and the extension and location of the implantation fossa. In *T. argo* the axonemal basis is located close behind the acrosomal vacuole in front of the implantation fossa. This arrangement completely differs from the above mentioned species. In the latter, the axonemal basis is located behind the implantation fossa which does not extend to the acrosomal vacuole, a situation typical for many other spider species (e.g., Alberti 1990; Alberti & Coyle 1991; Michalik et al. in press). The only exceptions in this respect known until now occur in the genera *Tetragnatha* and *Cyclosa* in which the implantation fossa also reaches the most anterior part of the

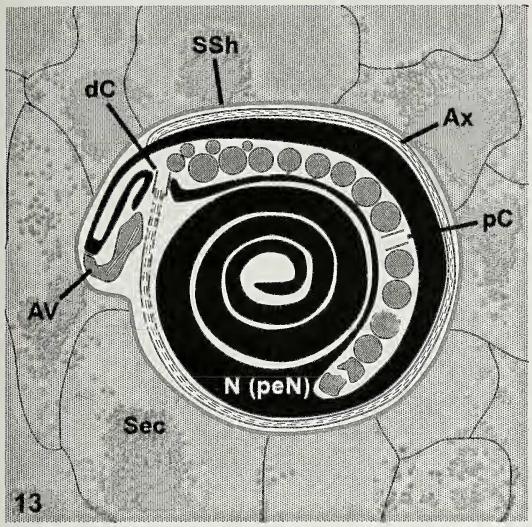


Figure 13.—Schematic reconstruction of a section of a mature spermatozoon in *Tidarren argo*. The nucleus coils as a spiral within the cell. The spermatozoon is surrounded by a thin secretion sheath and a considerable amount of dense secretion. Abbreviations: AV = acrosomal vacuole, Ax = axoneme, dC distal centriole, N (peN) = nucleus (postcentriolar elongation of nucleus), Sec = secretion, SSh = secretion sheath.

nucleus. However, in contrast to *T. argo*, in these species the axonemal basis is located in the posterior part of the implantation fossa as usual (Alberti 1990; Michalik et al. in press). Interestingly, in the theridiid spider *Neottiura bimaculata* (Linnaeus 1767) the position of the axonemal basis is similar to that seen in *T. argo*, but the nucleus and acrosomal vacuole are more compact, their shape therefore resembling that of other theridiid spiders. In *T. argo* the acrosomal vacuole shows an irregular arrowhead-shape and differs from the cylindrical or tube-like acrosomal vacuoles found in other theridiid species, e.g., *Argyrodes argyrodes* and *Theridion nigrovariegatum*. Of special interest is the dense secretion in which mature spermatozoa are embedded. Remarkably, the spermatozoa are loosely arranged in the palpal organ in comparison to other spider species, e.g., *Pachygnatha listeri* Sundevall 1830 (Michalik et al. in press). In this species no secretion was found and the spermatozoa have a thick protective secretion sheath. We suggest that in *T. argo* the protective function of the thick secretion sheath might be replaced by the copious secretion. Interestingly, the secretions in which the spermatozoa are embedded clearly differ between different species. In each of the theridiid spiders observed above we found a different structural aspect of the secretion. Since other spider families show different types of secretions (unpublished observations by the authors), a great diversity in this feature is revealed. This may reflect specific importance in the process of reproduction. As nothing is known about the function of male secretions and their possible role in the female genital system, this is an interesting topic for future research.

Tidarron argo possesses highly derivative and aberrant spermatozoa in contrast to other theridiid species, but more investigations on further theridiid species are needed to develop evolutionary scenarios and to clarify a possible phylogenetic and functional relevance of spermatological characters. Furthermore, it would be important to know more about the function and chemistry of the secretion in which the mature spermatozoa are embedded.

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